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(54) Title: METHOD AND APPARATUS FOR USE IN SOLID-PHASE PHYSICAL, CHEMICAL, BIOLOGICAL AND BIOCHEMI- CAL TECHNIQUES			
(57) Abstract <p>The method and apparatus which is suitable for performing a wide variety of processes involving the passage of a liquid through a solid-phase micro-particulate material (16) being provided within a plunger (10) that is capable of sliding movement within a well (1). Downward movement of the plunger (10) into the well (1) forces any liquid contained in the well to pass into the plunger and through the micro-particulate material (16). Reversing the movement of the plunger results in the liquid, now within the plunger, to be drawn back into the well. Thus, the liquid passes twice through the micro-particulate material and is caused to move without any external pumping or vacuum system. The method and apparatus is particularly suited to plural processing and to micro-chromatographic procedures.</p>			

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METHOD AND APPARATUS FOR USE IN SOLID-PHASE PHYSICAL, CHEMICAL, BIOLOGICAL AND BIOCHEMICAL TECHNIQUES

The present invention relates to a method of and apparatus for solid-
5 phase physical, chemical, biological and biochemical techniques. It is to be
understood that reference herein to solid-phase techniques is intended as
reference to reaction chemistry and analytical techniques in which a
process solution is passed through a porous micro-particulate solid.
Particularly, but not exclusively, the present invention relates a method and
10 apparatus for use in solid-phase extraction (SPE), commonly used for the
extraction of drugs, environmental compounds etc.; solid-phase synthesis,
commonly involving combinatorial chemistry, peptide synthesis etc.;
purification, involving ion-exchange; and biological processes such as
solid-phase based enzymatic, affinity, antibody, PCR reactions etc.

15 The extraction processes detailed above are commonly used, for
example, in the pharmaceutical and healthcare industries. Generally when
performing solid-phase techniques, the porous micro-particulate solid or
material is located in a cartridge through which the process solutions are
passed. Thus the cartridge acts both as a receptacle for the matrix and a
20 conduit for the liquid solutions and typically, solid-phase techniques employ
a plurality of individual cartridges simultaneously. In all cases the micro-
particulate solid is held in a cartridge that is fixed during the extraction
process. In other words both the cartridge and the solid within it are
stationary and it is the liquid solutions that move through the solid.

25 For example, SPE is performed to isolate substances of interest
present in various liquid samples (e.g. clinical samples such as plasma,
urine etc.). Using this technique, the liquid sample is passed through the
cartridge containing the appropriate micro-particulate SPE sorbent and the
substance of interest is selectively retained (i.e. extracted from solution)
30 due to its higher affinity with the sorbent compared with the solution. The
substance of interest is subsequently washed off the sorbent for isolation
and analysis. When this procedure is carried out the method generally

involves the following steps: each of the cartridges are pre-conditioned, washed, loaded with the liquid sample, washed again and then eluted using fixed volumes of appropriate solvents. In all of these steps, with the exception of the last, the solution is allowed to pass through the sorbent to waste. Only in the final step (when the substance is "washed off" the sorbent), sample elution, is the solution collected usually in a sample vial for analysis. Alternatively, SPE may be used to extract unwanted substances from a liquid sample. In this case the liquid sample is retained for analysis, after it has been flushed through the cartridge containing the micro-particulate solid, and the matrix-retained substances are subsequently disposed, i.e. the cartridges are thrown away. In all cases however, for each process step the liquid sample performs a single pass through the sorbent, i.e. uni-directional flow through the sorbent is performed.

15 The movement of the liquid sample through the micro-particulate solid is achieved using various techniques. For example, a vacuum may be applied to the downstream side of the micro-particulate solid to draw the liquid through the micro-particulate solid. Alternatively, the liquid sample may be pumped through the micro-particulate solid or be forced through by applying a positive pressure upstream of the micro-particulate solid. In 20 combinatorial chemistry the solution and the micro-particulate solid are sometimes agitated together in a suitable receptacle. Naturally, the use of pumps and other such peripheral equipment further increases the complexity and cost of the apparatus. Although these procedures are well established, conventional solid-phase techniques have a number of 25 inherent problems and limitations. These include inconsistent flow rates through the micro-particulate solid, poor or non-existent flow control, complex and expensive technologies and methodology limitations arising from the manner in which the techniques are operated. In particular, 30 although such procedures have been automated, the procedures remain slow and are usually performed one at a time and the necessary volumes of liquid required can produce deleterious effects when the eluate is

subsequently used in chromatographic analysis.

The present invention seeks to address the disadvantages of known solid-phase techniques and seeks to provide an improved method and apparatus for use in the performance of such techniques.

5 The present invention provides apparatus for use in solid-phase physical, chemical, biological and biochemical techniques comprising at least one well and a respective hollow plunger, the plunger containing a particulate material adjacent an aperture in the plunger and further including sealing means for forming a sliding seal between the plunger and
10 the interior of the well and drive means for moving the plunger into and out of the well, whereby liquid in the well is forced to flow into the plunger via the aperture and through the particulate material as the plunger is moved into the well and liquid in the plunger is forced to flow through the particulate material into the well via the aperture in the plunger when the
15 plunger is moved away from the bottom of the well.

Preferably, a plurality of wells and a corresponding plurality of plungers arranged in respective arrays are provided and the drive means controls movement of each of the plungers into and out of the wells simultaneously. Also, fluid channels can be provided around each of the
20 wells in the array to enable temperature control of the wells by means of a circulating fluid.

Furthermore, a sump or micro-vial may be provided integral with the base of the well forming the lowest accessible location for liquid within the well from which small volumes of liquid may be conveniently and
25 completely aspirated. Ideally, the base of the well is angled downwardly towards the sump to provide an easy downward path for liquid to the sump. Also, the plunger may include a nozzle communicating with the aperture in the plunger, the nozzle being shaped to fit into the sump in the base of the well whereby small volumes of liquid in the sump are aspirated
30 as the nozzle is inserted into the sump.

Preferably, the sealing means is in the form of a downwardly depending skirt located about the periphery of the plunger at a

predetermined height above the tip of the nozzle. The sealing skirt may be unitary with the plunger.

In a preferred embodiment, the drive means includes a support plate from which the plunger is suspended and ideally the drive means is
5 controlled by a motor such as an electric motor and/or by hydraulics. Alternatively, the support plate may provide a grip for manual control.

Also, the interior of the plunger may be connected to a manifold for controlling the pressure and content of the gas in the plunger and a casing may be provided within which the one or more wells and plungers are
10 located.

In a separate aspect the present invention provides a method of performing solid-phase physical, chemical, biological and biochemical techniques comprising the steps of: providing a particulate material in a hollow plunger having an aperture; charging a well with a process solution;
15 inserting the hollow plunger into the well; forming a sliding seal between the plunger and the well; forcing the process solution into the plunger through the aperture and through the particulate material by moving the plunger towards the base of the well; and forcing the solution back through the particulate material and into the well via the aperture in the plunger by
20 moving the plunger away from the base of the well.

Preferably, the above method steps are repeated with at least one further solution whereby washing, sample loading, and elution can be performed with the same plunger for each step. In this way solid-phase extraction can be performed.

25 Alternatively, the method steps are performed to introduce a chemical grouping to the particulate material and the steps are then repeated at least once to force a reaction solution through the particulate material including the chemical grouping.

In the case of SPE, affinity and biological processes the samples will
30 normally be aqueous based (e.g. water, aqueous buffers, urine, plasma, blood etc.). In the case of solid-phase chemistry the sample may be either an aqueous solution or an organic solution.

Thus, in contrast to conventional apparatus and methods where both the receptacle and the micro-particulate material are stationary, with the present invention the particulate material is moved as part of the plunger so as to force liquid through it. The invention affords significant advantages to the varied processes that can be performed using this apparatus and method. For example, the invention allows for single, multiple or continuous cycle operation, it also allows accurate and precise flow control, multiple parallel processing and in general is easier to use than conventional apparatus.

10 The double pass of the solution through the particulate material improves reproducibility, reaction completion and extraction efficiencies. Also, as the plungers all move simultaneously, the flow rate of solution through the disc can be more accurately controlled. The sumps at the bottom of the wells also function as autosampler vials thereby avoiding the need for the elute to be transferred to a separate vial before analysis. The sump in combination with the sealing skirt on the plunger also ensure that all of the solution in the well is passed through the solid particulate disc each cycle and enables very small volumes of liquid to be used with the apparatus.

20 In a separate further aspect the present invention provides chromatographic apparatus comprising a substantially vertical elongate well containing a movable cartridge of chromatographic material, sealing means for forming a liquid tight seal between the sides of the cartridge and the inner walls of the well, driving means for moving the cartridge between upper and lower positions within the well, and an outlet control for collecting liquid from above the cartridge in the well whereby the cartridge is arranged to move through a liquid introduced into the well with the liquid that has passed through the cartridge being removed and selectively collected.

Also, the present invention provides a method of performing chromatographic separation comprising the steps of: introducing a cartridge of chromatographic material into a substantially vertical elongate well; forming a seal between the sides of the cartridge and the walls of the well;

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supplying a liquid to the base of the well; driving the cartridge from a first upper position in the well to a second lower position within the well thereby forcing the liquid to pass through the chromatographic material; and selectively collecting liquid from above the cartridge.

5 Additionally, in a further aspect the present invention provides apparatus for use in solid-phase physical, chemical, biological and biochemical techniques comprising at least one well and a respective hollow plunger, the plunger having sealing means for forming a seal with the walls of the well and a downwardly directed nozzle communicating with
10 the interior of the plunger and the well including a sump at its base conformed to closely fit about the nozzle of the plunger whereby small volumes of liquid can be drawn from the sump into the plunger by movement of the plunger towards the base of the well and substantially wholly aspirated.

15 Embodiments of the present invention will now be described by way of example only with reference to the accompanying drawings, in which:

Figure 1 is a schematic diagram of solid-phase process apparatus in accordance with the present invention;

20 Figures 2a, 2b, 2c, 2d and 2e are schematic diagrams representing different stages of a process method in accordance with the present invention;

Figure 3 shows schematically an array of the solid phase process apparatus of Figure 1 with the plungers partially inserted into the wells, in accordance with the present invention;

25 Figure 4 shows a machine incorporating the apparatus of Figure 3 for performing the method, in accordance with the present invention;

Figures 5 illustrates chromatographic apparatus in accordance with the present invention, and

30 Figures 6a through to 6m illustrate a chromatographic method, in accordance with the present invention.

The apparatus shown schematically in Figure 1 substantially comprises a well 1 and a hollow plunger 10. The well 1 is substantially

cylindrical in construction and at an upper end the well has a rim 2 defining an opening 3 to the interior 4 of the well. Preferably, the inner wall of the rim 2 is frusto-conical in shape having a greater diameter at the outermost periphery of the well. The inner wall of the rim 2 therefore acts as a cam surface or guide surface leading to the interior of the well. The base 5 of the well is closed so that a liquid may be contained in the interior 4 of the well. The base 5 is shaped so as to generally conform to the outer shape of the plunger 10, which will be described in greater detail below. As shown in Figure 1, the base 5 has a downwardly depending generally conical inner surface that terminates in a centrally located sump 6 or micro-vial. Although the sump 6 is shown in the figures with a flat bottom, the sump 6 may alternatively terminate in a centrally located conical base.

The plunger 10 has a generally cylindrical hollow body 11 having an outer diameter slightly less than the diameter of the interior of the well 1. The plunger 10 is open at its upper end and terminates at its lower end 12 in a downwardly depending spigot 13 or nozzle that contains a narrow open channel 14 which communicates with the interior of the plunger body 11. The outer surface of the lower end 12 of the plunger 10 between the spigot 13 and the walls of the cylindrical body 11 is generally conical in shape and substantially corresponds in shape with the inner surface of the base 5 of the well. Moreover, the height of the spigot 13 is substantially equal to the depth of the sump 6 of the well. The lower end of the plunger 10 and the interior of the base 5 of the well are thus shaped so that when the plunger is positioned in the interior of the well, the lower end 12 of the plunger engages with and conforms to the interior surface of the base 5 of the well. The fluid passage or channel 14 tapers outwardly towards the interior of the plunger 10. In this way liquid rising up the channel 14 spreads to cover the inner floor of the plunger and "floods" the area immediately above the floor of the plunger before extending upwards.

Additionally, a downwardly depending skirt 15 extends outwardly from the periphery of the plunger body 11. The skirt 15 is positioned slightly above the lower end 12 of the plunger 10 and is arranged to

engage with the interior walls of the well 1 to form a liquid/air tight seal therewith. Where a sealing skirt is not provided the plunger is designed to form an interference fit with the walls of the well to ensure that liquid contained in the well is not drawn up between the walls of the plunger and the well during movement of the plunger within the well. The skirt 15 may be integral with the plunger or may be secured to the outer wall of the plunger in a peripheral groove.

Immediately above the spigot 13 in the interior of the plunger a disc of a solid micro-particulate material 16 is provided and is preferably held in position by means of upper and lower locating rings 17 or porous disc retainers. The disc 16 may be made from a variety of known materials suitable for use in SPE, affinity processes, for multiple chemical reactions of the type performed in combinatorial chemistry or for enzyme reactions and other biological process. For example the disc may be made of bonded silicas or polymers such as polystyrene divinyl benzene.

As may be seen more clearly from Figures 3 and 4, a plurality of wells may be connected together to form an array, for example a 6 X 4 array. It will of course be understood that alternative arrangements of the wells in an array may also be employed as appropriate. In Figures 1 and 4 the wells are shown connected together by means of flanges extending radially from the rims of each of the wells to form a continuous supporting structure. On the other hand, the wells may be formed as a regular array of apertures in a solid block.

Preferably, a plurality of plungers may also be connected together to form a plunger array corresponding to the array of wells whereby the plurality of plungers and respective wells may be used simultaneously for plural processing. Ideally, the plungers are connected together by means of a support plate 18. The support plate 18 has an array of holes from which the plungers individually hang. Each of the holes in the support plate 18 has a diameter slightly greater than the outer diameter of the plunger and has a ring-shaped recess for engaging with a radially outwardly extending lip 19 on the upper edge of the plunger.

The support plate 18 allows the escape/ingress of air into the top of the plunger whilst fluids are moving into and out of the plunger, as will be described in greater detail below. Alternatively, the interiors of the plungers may be connected to a manifold (not shown) to enable air to be removed and/or and alternative gas introduced. This procedure is particularly useful in solid-phase chemistry where it may be necessary to purge the insides of the plungers with an inert gas such as argon.

To ensure accurate alignment of each plunger in an array of plungers in its respective well, the frusto-conical inner surface of the well rims 2 engage with and guide the lower ends 12 of the plungers into their respective wells. Also, the plungers need not be permanently fixed to the support plate 18 and instead may be capable of limited sideways movement relative to the support plate 18 so that an individual plunger can be deflected by the rim of its respective well and so self-centre itself within the well. In this way alignment of the plungers within the wells may be optimised.

Typically the plunger 10 may be made from an inert polymer such as polypropylene and is made by injection moulding. Alternatively, the plunger may be made from glass with the skirt 15 consisting of a radial polypropylene lip secured in a groove in the outer wall of the glass plunger. Also, the plunger may include a ptfе or glass liner in its inner cavity. The wells may also be made from a similar plastics material by injection moulding either as individual wells as shown in Figure 3 or as an array of apertures in a block. Alternatively, the wells may be made from glass or metal with suitable inert liners to the well cavity. Where the wells are constructed as individual wells connected together by means of their rims, the open spaces between the individual wells may be used to circulate a fluid that may be used to heat or cool liquid contained within the wells. Ideally, the open spaces between the wells are divided up to provide labyrinthine flow between the wells.

Ideally, as shown in Figure 4, the wells and plungers are provided within an air-tight casing 20 so that the environment in which the chemistry

or other processes are performed may be wholly controlled. To enable access to the wells and plungers the casing 20 preferably includes a movable cover on hinges or the like. Also, the substantially vertical movement of the support plate 18 from which the plungers hang may be performed manually or may be controlled by means of electrically operated pneumatic/hydraulic arms. As seen in Figures 3 and 4, the supporting structure of the wells includes side walls 21 that terminate at their low edges in lips 22. The lips 22 project outwardly to engage with respective rails 23 provided within the casing 20. The rails are provided to guide the positioning of the array of wells within the casing. The rails 23 also engage the lips 22 to prevent the wells being lifted upwards when the plungers are raised from their lowest position within the wells. Similar guide rails 24 are provided to receive the support plate 18 of the plungers. The guide rails 24 ensure accurate positioning of the plungers with respect to the wells and secure the support plate 18 to the drive member that controls upward and downward movement of the plungers. It is envisaged that the wells may also include a floor, connected to the side walls 21 to define the cavity in which a temperature controlled fluid may be circulated. The floor may be resilient so that slight downward movement of the wells, when the plungers reach the bottoms of the wells, can be accommodated.

In Figure 4 two individual sets of arrays are shown in the casing 20. It is envisaged though that the apparatus may be used in a continuous operational mode. To achieve continuous operation, a single array of plungers is provided in combination with a series of separate arrays of wells mounted on a moving support such as a conveyor belt. Rails similar to those shown in Figure 4 would be used to guide the arrays of wells as they travel along the conveyor belt. Each of the arrays of wells represents a separate process station at which a particular step in the process is performed. For example, the first stage may consist of an array of wells in which wash solutions are provided. The array of plungers is lined up with the first well array and the plungers are lowered into the wells in the same manner as described above. The plungers are then raised again and the

conveyor belt is moved on to present the array of plungers with the next process station at which a new well array is provided but this time the wells may contain a sample solution, and so on. Although the continuous operation has been described by reference to discrete well arrays it will of course be apparent that a single very large scale array of wells may be employed with a sub-set of the array representing a particular process step. In this alternative arrangement the array of plungers rather than the wells may be moved to align up with any particular grouping of wells for a particular process to be performed. It will be immediately evident that with such an arrangement any of the various physical, chemical, biological or biochemical techniques referred to above may be performed with a continuous series of plunger arrays passing sequentially along the conveyor belt.

Operation of the SPE apparatus will now be described with reference to Figures 2a - 2e.

Figure 2a: the well 1 is charged with a sample solution 30 that collects in the sump 6 and the lower region of the well. The plunger 10 is then inserted into the interior of the well forming a seal with the inner walls of the well by means of the skirt 15 and the plunger is moved downwards into the well. As the skirt 15 forms an air-tight seal with the inner walls of the well, whilst the plunger is forced further into the well, air within the well is forced through the channel 14 in the spigot of the plunger and is released via the plunger body.

Figure 2b: Once the tip of the spigot 13 encounters the sample solution within the well, further downward movement of the plunger causes the solution to be drawn up the channel 14 in the spigot. The solution is forced through the solid particulate disc 15 and collects in the plunger above the disc. It is believed that the downward movement of the plunger applies pressure to a pocket of air between the top of the liquid in the well and the skirt 15. The greater the downward pressure applied to the plunger, the air pocket is believed to be partially compressed and forces the skirt 15 into even closer sealing engagement with the inner walls of the well. The air

pocket may be described as establishing a "head of pressure" acting on the surface of the liquid.

The pressure applied to the air pocket is transmitted to the liquid in the well and so causes the liquid to rise up the spigot or nozzle into the plunger. This means that as soon as the spigot 13 comes into contact with the surface of the liquid, the liquid flows up through the channel 14 into the plunger. This in turn results in only the very end region of the spigot coming into contact with the liquid. This minimises the exposure of the head of the plunger to the potentially corrosive effect of certain solvents employed in the processes for which this apparatus is particularly suited. Figure 2c: As more of the solution is drawn up through the channel 14 in the spigot, the remaining solution in the well collects in the sump 6. As the spigot 13 is shaped to fit within the sump the solution continues to be drawn up through the channel 14 and through the disc until no solution remains. Moreover, because the skirt 15 forms an air-tight seal with the walls of the well and the position of the skirt 15 with respect to its height above the spigot tip is selected to define a volume greater than the volume of the sump, the final downward movements of the spigot into the sump causes any remaining air within the well also to be forced through the channel 14. Thus, the volume of air displaced by the movement of the plunger into the well, once the tip of the spigot has encountered the surface of the liquid, is greater than the total of the volume of the liquid and the volume of the channel in the spigot. In this way, the final drops of the solution are forced from the channel through the disc 15 into the plunger body.

Figure 2d: The plunger 10 is then drawn upwards out of the well 1. Upward movement of the plunger causes the liquid within the plunger body to be sucked back through the disc 15, down the channel 14 into the well 1. Upward movement of the plunger is believed to create a partial vacuum under the skirt in the well which "sucks" the liquid back into the well.

Figure 2e: upward movement of the plunger is continued until all of the liquid within the plunger is returned to the well and the plunger clears to the

top of the well.

From the above it will be appreciated that the solution is forced twice through the solid particulate matrix disc 15, unlike conventional apparatus that rely upon a single pass of the liquid through the matrix. Moreover, unlike conventional apparatus where the solid particulate matrix is stationary, the solid particulate matrix disc moves as part of the plunger within the well and the flow of liquid through the matrix is controlled by means of that movement of the plunger within the well: no mechanical agitation, additional pressurisation or an applied vacuum of the liquid is required as the plunger acts as its own "pump" for the circulation of the liquid.

Although the apparatus has been described above with reference to SPE processes, four processes are particularly suited to being performed using the apparatus described above. i) Solid-phase extraction; ii) affinity processes; iii) solid-phase chemistry or combinatorial chemistry; and iv) enzyme and other biological processes.

Solid-Phase Extraction

During this procedure the process solutions are dispensed into the individual wells of a multiple well array. The array of wells is then located in position under the corresponding array of plungers, either manually or automatically, and the individual wells are aligned with respective plungers overhead.

The plungers are then lowered into the wells and travel to the bottom of the wells forcing the process solutions through the solid particulate disc into the body of the plunger. The rate of downward movement of the plungers is determined on the process requirements and is preferably automatically controlled. This represents the first pass of the liquid through the solid particulate disc.

The plungers are then withdrawn from the wells thereby causing the liquid within the plunger body to return through the solid particulate disc into the well. This represents the second pass of the liquid through the disc.

As the skirt 15 forms a seal with the walls of the well, the upward movement of the plunger within the well creates a partial vacuum in the base of the well that acts to suck the liquid back into the well. The movement of the plunger alone is sufficient to ensure the return of the liquid into the well. In certain circumstances this may be pressure assisted by applying pressure to the liquid within the plunger via the manifold.

It will of course be apparent that the above procedure could be repeated a number of times where necessary with there being two passes of the liquid through the disc for each cycle. In all cases however, the solution returns to the well at the end of the cycle.

During the movement of the process solutions through the solid particulate disc, the specific process associated with each solution takes place. Thus, when the well contains the pre-condition solution, the solid particulate disc is pre-conditioned. Likewise, when the wash, study sample, second wash and elution solutions are introduced into the well the wash, sample extraction, second wash and sample elution steps are performed respectively.

In this manner, all the separate steps of solid-phase extraction can be performed, analogous to the conventional method and the material of the disc may be conventional for example bonded silicas that are spherical or irregularly shaped particles of the order of 1-100 microns in diameter. The chemical grouping of the material usually has a high carbon number e.g. $(CH_2)^{17}-CH_3$. These materials are lipophilic and can "bind" a wide variety of chemical compounds. Alternatively, the chemical grouping may be aromatic, or a charged ion species to enable interaction with cationic or anionic species. The micro-particulate material may alternatively be a polymer such as polystyrene divinyl benzene.

In the overall process, five separate well arrays are generally used. After each cycle the individual wells contain the solutions that have passed through the solid particulate disc. The final well in the process contains the eluted sample in the elution liquid. This would normally be analysed directly (e.g. chromatographically - see below). The integral micro-vial also

assists in the aspiration of the eluate from the well once the process is completed as the array of wells is particularly suited to use with conventional X-Y autosamplers.

In most cases the volume of the elution aliquot would normally be less than the volumes used in the previous steps. If this volume is less than the sample volume then the sample will be more concentrated in the elution liquid than in the original sample. Thus, if the concentration in the original sample were 100 units and the elution volume was 25% of the sample volume, the concentration of the compound of interest in the elution liquid would be 400 units (assuming 100% extraction/elution of the sample).

The small elution volume has presented problems with conventional apparatus in the past in terms of aspirating the eluate from the well or test tube. However, by means of the sump and spigot arrangement of the apparatus described above the sump acts as an integral micro-vial from which the sample can be aspirated.

Affinity Processes

Affinity processes are performed to isolate specific molecules in solution or to purify the solution. This is achieved by attaching selected affinity molecules or affinity sites such as anti-bodies to a solid support material, similar to the matrix used in SPE. The selected affinity species are chosen on the basis of the specific molecule in solution with which they are required to interact. The specific molecule is subsequently eluted from the particulate affinity medium. Thus, affinity techniques using solid micro-particulate support material, made from for example porous polymer beads to which affinity species can be bonded, can be performed generally using the SPE procedure described above, albeit that the process solutions will be different.

Thus, at some point in the process specific molecules would be removed from sample solutions due to the affinity of the molecule for a chemical grouping on the solid micro-particulate disc and at a later point these molecules would be desorbed for use in a later separate process.

Solid-Phase Synthesis

The process for solid-phase chemistry, e.g. combinatorial chemistry, would be carried out in a similar manner to that described above. In this case though the process solutions would normally be solutions of appropriate chemical reactants or wash solutions and the material of the micro-particulate disc may be solid-phase synthesis polymer beads of 1-500 microns. Usually, the polymer derivative chosen would be appropriate for the reaction being performed. For example, a resin with carboxyl groups on the surface might be appropriate for a reaction with amines thereby forming a stable amide linkage that would anchor the first building block of the resin. Polystyrene is often used as the base backbone of these polymer types.

Thus, as the solutions of reactants pass through the solid particulate disc, chemical species attached to the matrix of the disc would be chemically modified in some way. By this process, a wide range of different chemical reactions can be carried out simultaneously using the well and plunger arrays. The capacity of the system to allow repeated plunger cycles would be useful here as the solutions can be passed through the disc many times to ensure the reaction is completed.

For these types of chemical reaction the ability to control the temperature of the wells during the reactions is important. Hence, as mentioned above, a fluid such as water at a predetermined temperature may be circulated around the outside of the wells. The temperature of the water is preferably automatically controlled using conventional techniques and apparatus. Moreover, by connecting the interior of the plungers to a manifold, the exposure of the solutions to inert environments, such as argon, can additionally be performed and again automatically controlled.

30 Biological and Biochemical Processes

Biological and biochemical processes, for example, enzyme reactions, PCR reactions, nuclear genetic processes, cellular biological

organell processes, metabolic reactions, biosynthesis processes, biodegradation processes, toxicity processes and bacterial and viral processes may all be performed in a similar manner to that described above. In this case the process solutions would normally be aqueous
5 based. The micro-particulate material may be made from a wide variety of porous beads such as lipophilic bonded silicas of between 1 and 500 microns. Alternatively, enzymes can be chemically attached through an appropriate link (amine or carboxyl) to these materials or polymer beads. Imprint polymers are increasingly used in conventional processes where
10 the polymers have an active site chemically designed into the structure of the polymer.

Thus, as the solutions appropriate for the aforementioned biological and biochemical processes pass through the solid-particulate disc, these processes take place. In this way, a wide range of different biological and
15 biochemical processes can be performed simultaneously using the well and plunger arrays. The capacity of the system to allow repeated plunger cycles would be useful here as the solutions can be passed through the disc many times to ensure the process is completed.

For these type of biological and biochemical processes the ability to
20 control the temperature of the wells during the process is important and the apparatus and method described earlier for temperature control is preferably employed.

It can thus be seen that the apparatus and method described above afford various advantages over existing apparatus and methods. In
25 particular, it is possible for very small quantities of a sample solution to be aspirated from the sump or micro-vial at the bottom of the well. The micro-vial is also particularly useful in permitting aspiration of such small quantities of liquid by an autosampler, for example.

Furthermore, the apparatus enables plural processing to be
30 performed which can significantly reduce the overall time in performing relevant procedures. The plural processing also ensures improved reproducibility and improved efficiency and ensures that the flow rates of

solutions through the solid particulate disc can be more consistently and accurately controlled as all the plungers are moved simultaneously into and out of their respective wells.

As the plunger moves within the well thereby causing the liquid
5 within the well to pass through the micro-particulate solid, the need for complex and costly independent pumping systems is obviated and the apparatus is extremely simple to use. The movement of the plunger within the well also affords an inherent flow control and the facility to re-cycle solutions through the micro-particulate material ensures process
10 completion and improved data quality.

The apparatus is also allows for multiple mode processing. Thus, after a synthesis or biochemical step, the array of plungers can be replaced with others containing SPE sorbent thereby enabling an SP extraction step to be performed in the same format. This reduces the amount of
15 equipment required in a lab and avoids the need to use different apparatus for different processes. This in turn enables rapid plural processing in a manner that has not been previously achieved.

The above method is also suitable for a chromatographic technique. As shown in Figure 5 the chromatographic apparatus is substantially the
20 same as described earlier. The apparatus includes one or more wells 30 having a structure substantially identical to that described in Figures 2a to 2e including micro-vial 31 in the base of the well 30. Each well has an associated hollow plunger or cartridge 32 with a nozzle 33 shaped to conform to the internal walls of the bottom of the well and micro-vial 31.
25 The plunger also includes a seal for sealing with the walls of the well. As before, the seal is preferably in the form of a peripheral downwardly depending skirt. In this case, instead of a disc-like sample of micro-particulate material, suitable for extraction, solid-phase synthesis, biological, affinity or other processes as described above, the material 34
30 would be suitable for chromatographic separation. In addition, the amount of chromatographic material is preferably increased and may possibly extend the entire length of the cartridge. The cartridge is closed at the top,

with an outlet aperture to allow movement of the chromatographic mobile phase out of the cartridge 32 during the chromatographic process. This would pass out through an outlet conduit 35.

Where arrays of wells and plungers are employed, plural
5 chromatography may be carried out, analogous to the plural processing previously described. Thus, during the process, the array of cartridges would be moved simultaneously downwards into their respective wells each of which being suitably charged with chromatographic mobile phase. During this process, the mobile phase would pass upwards through the
10 cartridges 32, where the separation would take place. As this progressed, the mobile phase would leave via the outlet conduit 35. The component (s) of interest would thus be separated in the column within the cartridge 32 and at a certain time (the elution time) and a predetermined position of the cartridge within the well, the component (s) would elute out of the cartridge.
15 This portion of the sample might be collected for further analysis. Before and after this step, the elute might drain to waste. Only when the compound of interest is eluting from the cartridge would collection normally be required.

As previously mentioned, this allows simultaneous multiple (plural)
20 chromatographic analysis. However, whereas other known plural systems require plural pumping systems, the apparatus described above does not. In this case, the action of the plunger as it moves into the well drives the mobile phase through the column. The movement of the plunger also controls the rate. For some applications the rate might be slow, for others,
25 it might be fast. Alternatively, the flow rate might be varied throughout the run.

Turning now to Figures 6a to 6m the apparatus can be operated as follows:

- i) An array of wells is loaded with mobile phase 36 and the
30 chromatographic cartridges are preconditioned (with mobile phase) by moving the cartridges into and out of their respective well, previously charged with mobile phase (Figures 6a-6e). Although

- some of the mobile phase returns to the well as the plunger is being drawn up out of the well, there would be sufficient residual liquid on the surface of the chromatographic material to retain its chromatographic activity (i.e. it would not dry out). It would not dry out since the volume of air passing through the cartridge when it is withdrawn from the well would not be sufficient to dry the material.
- 5
- ii) The sample of interest 37 is then added to a new array of wells for chromatographic analysis. This would normally be a small sample, appropriate for a small chromatographic column. For example, it might be 5-10 microlitres, diluted to perhaps 50 microlitres so that it could be readily aspirated (Figure 6f).
- 10
- iii) The added sample for analysis collects in the micro-vial at the base of each well. The same cartridges are then moved into the new array of wells and the sample is forced up into the chromatographic material (Figures 6g and 6h). During this process, the substances of interest become bound to the chromatographic material (i.e. would be pulled out of the sample solution). The cartridge is withdrawn and the sample (minus the compound of interest, which is bound to the chromatographic material) returns to the well (Figures 6i and 6j).
- 15
- 20 If necessary, the cartridge might be pushed into and out of the well repeatedly so as to circulate the sample into and out of the column more than once. This would ensure that all the compound of interest is completely pulled out of the sample.
- iv) Finally, a new array of wells is charged with mobile phase 38 (Figure 6k) and the same cartridges are slowly moved into the mobile phase in the new array of wells. As this progresses, the chromatographic separation takes place as previously described (Figures 6l and 6m). Initially, the chromatographic mobile phase coming out of the cartridge would be sent to waste. Then, as the compound (s) of interest is eluted, the sample would be collected into a suitable receptacle. This would apply to every cartridge. Therefore, if there were 48 cartridges, there would be 48 outlets and 48 separations
- 25
- 30

could be achieved simultaneously.

The apparatus described above lends itself to rapid micro-scale chromatographic separations, relevant to many innovations today such as miniaturisation. Also, it allows simple, throwaway or disposable
5 chromatographic columns to be used. No other known technologies allow this. Moreover, flow rates would be very controllable and the apparatus is very time and cost saving as well as simple. It should be further noted that unlike conventional chromatographic injection systems (which normally involve loading a sample loop and switching on-line with the
10 chromatographic column), the apparatus and method described above involves the direct application of the sample into the chromatographic material using the movement of the cartridge within the well as a 'pseudo-injection' technique.

Although this process may involve higher pressures than for the
15 processes described earlier, due to there being more material in the cartridge, the design of the skirt-like seal is such that as the pressure increases, the skirt is forced harder against the walls of the well, thus improving the seal. Furthermore, as with the apparatus of Figures 1 to 4, because of the arrangement of the plunger nozzle and the micro-vial, very
20 small volumes of liquid can be substantially completely aspirated which is important with chromatographic apparatus of this type. In the case of the loading of the sample using the apparatus described all of the sample volume is aspirated into the chromatographic material.

It will be immediately apparent that as with the apparatus of Figures
25 1 to 4, unlike conventional apparatus, it is the solid particulate material that moves through the solution. Moreover, here too the movement of the cartridge towards the base of the well forces the solution into the plunger cavity without the need for injection or pumping of the solution.

CLAIMS

1. Apparatus for use in solid-phase physical, chemical, biological and biochemical techniques comprising at least one well and a respective
5 hollow plunger, the plunger containing a particulate material adjacent an aperture in the plunger and further including sealing means for forming a sliding seal between the plunger and the interior of the well and drive means for moving the plunger into and out of the well, whereby liquid in the well is forced to flow into the plunger via the aperture and through the
10 particulate material as the plunger is moved into the well and liquid in the plunger is forced to flow through the particulate material into the well via the aperture in the plunger when the plunger is moved away from the bottom of the well.
- 15 2. Apparatus as claimed in claim 1, comprising a plurality of wells and a corresponding plurality of plungers arranged in respective arrays and wherein the drive means controls movement of each of the plungers into and out of the wells simultaneously.
- 20 3. Apparatus as claimed in claim 2, wherein guide rails are provided for engaging with one or more edges of the array of wells and the array of plungers whereby the rails engaging the array hold the array in place as the plungers are raised upwards within the wells.
- 25 4. Apparatus as claimed in either of claims 2 or 3, wherein fluid channels are provided around each of the wells in the array to enable temperature control of the wells by means of a circulating fluid.
- 30 5. Apparatus as claimed in any one of claims 1 to 4, wherein a sump is provided in the base of the well and provides the lowest accessible location for liquid within the well from which small volumes of liquid may be aspirated.

6. Apparatus as claimed in claim 5, wherein the base of the well is angled downwardly towards the sump.

5 7. Apparatus as claimed in claims 5 or 6, wherein the plunger includes a nozzle communicating with the aperture in the plunger, the nozzle being shaped to fit into the sump in the base of well whereby small volumes of liquid in the sump are aspirated as the nozzle is inserted into the sump.

10 8. Apparatus as claimed in any one of the preceding claims, wherein the sealing means is in the form of a downwardly depending skirt located about the periphery of the plunger above the aperture.

9. Apparatus as claimed in claim 8, wherein the sealing skirt is unitary
15 with the plunger.

10. Apparatus as claimed in any one of the preceding claims, wherein the plunger is made from an inert material.

20 11. Apparatus as claimed in any one of the preceding claims, wherein the drive means includes a support plate from which the plunger is suspended.

12. Apparatus as claimed in claim 11, wherein the drive means is
25 controlled by an electric motor and/or by hydraulics.

13. Apparatus as claimed in any one of the preceding claims wherein the interior of the plunger is connected to a manifold for controlling the pressure and content of the gas in the plunger.

30

14. Apparatus as claimed in any one of the preceding claims, further including a casing within which the one or more wells and plungers are

located.

15. Method of performing solid-phase physical, chemical, biological and biochemical techniques comprising the steps of: providing a particulate
5 material in a hollow plunger having an aperture; charging a well with a sample solution; inserting the hollow plunger into the well; forming a sliding seal between the plunger and the well; forcing the sample solution into the plunger through the aperture and through the particulate material by moving the plunger towards the base of the well; and forcing the solution
10 through the particulate material into the well via the aperture in the plunger by moving the plunger away from the base of the well.

16. Method as claimed in claim 15, wherein the steps are repeated with at least one further solution whereby washing, sample loading, and elution
15 can be performed with the same well and plunger for each step.

17. Method as claimed in claim 16, wherein solid-phase extraction is performed.

20 18. Method as claimed in claim 15, wherein the steps are performed to introduce a chemical grouping to the particulate material and the steps are then repeated at least once to force a reaction solution through the particulate material including the chemical grouping.

25 19. Method as claimed in claim 18, wherein a specific affinity species is introduced into the particulate material.

20. Method as claimed in claim 18, wherein at least one enzyme is introduced into the particulate material.

30

21. Chromatographic apparatus comprising a substantially vertical elongate well containing a movable cartridge of chromatographic material,

sealing means for forming a liquid tight seal between the sides of the cartridge and the inner walls of the well, driving means for moving the cartridge between upper and lower positions within the well, and an outlet control for collecting liquid from above the cartridge in the well whereby the
5 cartridge is arranged to move through a liquid introduced into the well with the liquid that has passed through the cartridge being removed and selectively collected.

22. Chromatographic apparatus as claimed in claim 21, wherein the
10 cartridge includes a downwardly projecting nozzle having a channel leading to the interior of the cartridge and the well includes a micro-vial at its base whereby the nozzle is conformed to closely fit within the micro-vial thereby enabling small quantities of liquid to be drawn into the cartridge.

15 23. A method of performing chromatographic separation comprising the steps of: introducing a cartridge of chromatographic material into a substantially vertical elongate well; forming a seal between the sides of the cartridge and the walls of the well; supplying a liquid to the base of the well; driving the cartridge from a first upper position in the well to a second lower
20 position within the well thereby forcing the liquid to pass through the chromatographic material; and selectively collecting liquid from above the cartridge.

24. A method of performing chromatography as claimed in claim 23,
25 wherein the liquid is collected in dependence on the position of the cartridge within the well.

25. A method of performing chromatography as claimed in either of claims 23 or 24, further including the preliminary steps of supplying a
30 sample solution to the well and loading the chromatographic material in the cartridge with the sample by driving the cartridge from an upper position to a lower position in the well thereby forcing the sample solution to pass into

the chromatographic material.

26. Apparatus for use in solid-phase physical, chemical, biological and biochemical techniques comprising at least one well and a respective
- 5 hollow plunger, the plunger having sealing means for forming a seal with the walls of the well and a downwardly directed nozzle communicating with the interior of the plunger and the well including a sump at its base conformed to closely fit about the nozzle of the plunger whereby small volumes of liquid can be drawn from the sump into the plunger by
- 10 movement of the plunger towards the base of the well and substantially wholly aspirated.

27. Apparatus as claimed in claim 26, wherein the sump is arranged to hold up to 200 microlitres.

15

28. Apparatus as claimed in either of claims 26 or 27, wherein the location of the sealing means on the plunger is selected whereby the volume of air displaced by the movement of the plunger into the well, from an upper position where the tip of the nozzle is at the entry to the sump to a
- 20 lower position where the tip of the nozzle is at the base of the sump, is greater than the total of the volume of the liquid in the sump and the volume of a channel in the nozzle providing communication between the interior of the plunger and the well.

25

AMENDED CLAIMS

[received by the International Bureau on 27 April 1998 (27.04.98);
original claims 1-28 replaced by amended claims 1-25 (5 pages)]

1. Apparatus for use in solid-phase physical, chemical, biological and
biochemical techniques comprising at least one well and a respective
5 hollow plunger, the plunger having sealing means for forming a seal with
the walls of the well and a downwardly directed nozzle communicating with
the interior of the plunger, and wherein the well including a sump at its base
conformed to closely fit about the nozzle of the plunger whereby small
volumes of liquid are drawn from the sump into the plunger by movement of
10 the plunger towards the base of the well and are substantially wholly
aspirated.
2. Apparatus as claimed in claim 1, wherein the location of the sealing
means on the plunger is selected such that the volume of air displaced by
15 the movement of the plunger into the well, from an upper position where
the tip of the nozzle is at the entry to the sump to a lower position where
the tip of the nozzle is at the base of the sump, is greater than the total of
the volume of the liquid in the sump and the volume of a channel in the
nozzle providing communication between the interior of the plunger and the
20 well.
3. Apparatus as claimed in either of claims 1 or 2, further including
drive means for controlling movement of the plunger into and out of the
well, whereby liquid in the well is forced to flow into the plunger via the
25 nozzle as the plunger is moved into the well and liquid in the plunger is
forced to flow into the well via the nozzle when the plunger is moved away
from the bottom of the well.
4. Apparatus as claimed in claim 3, comprising a plurality of wells and
30 a corresponding plurality of plungers arranged in respective arrays and
wherein the drive means controls movement of each of the plungers into
and out of the wells simultaneously.

5. Apparatus as claimed in claim 4, wherein guide rails are provided for engaging with one or more edges of the array of wells and the array of plungers whereby the rails engaging the array hold the array in place as the plungers are raised upwards within the wells.

6. Apparatus as claimed in either of claims 4 or 5, wherein fluid channels are provided around each of the wells in the array to enable temperature control of the wells by means of a circulating fluid.

10

7. Apparatus as claimed in any one of the preceding claims, wherein the sump provides the lowest accessible location for liquid within the well from which small volumes of liquid may be aspirated.

8. Apparatus as claimed in claim 7, wherein the base of the well is angled downwardly towards the sump.

9. Apparatus as claimed in any one of the preceding claims, wherein the sealing means is in the form of a downwardly depending skirt located about the periphery of the plunger above the nozzle.

10. Apparatus as claimed in claim 9, wherein the sealing skirt is unitary with the plunger.

11. Apparatus as claimed in any one of the preceding claims, wherein the plunger is made from an inert material.

12. Apparatus as claimed in any one of the preceding claims, wherein the plunger contains a particulate material adjacent an aperture in the plunger that communicates with the nozzle.

13. Apparatus as claimed in any one of claims 3 to 12, wherein the drive

means includes a support plate from which the plunger is suspended.

14. Apparatus as claimed in claim 13, wherein the drive means is controlled by an electric motor and/or by hydraulics.

5

15. Apparatus as claimed in any one of the preceding claims wherein the interior of the plunger is connected to a manifold for controlling the introduction and removal of fluid into the plunger.

10 16. Apparatus as claimed in any one of the preceding claims, further including a casing within which the one or more wells and plungers are located.

15 17. Apparatus as claimed in any one of the preceding claims, wherein the sump is arranged to hold up to 200 microlitres.

18. Chromatographic apparatus comprising at least one well and a respective cartridge containing chromatographic material, the cartridge having sealing means for forming a seal with the walls of the well and a
20 downwardly directed nozzle communicating with the interior of the cartridge and wherein the well includes a sump at its base conformed to closely fit about the nozzle of the cartridge whereby liquid in the well is drawn into the cartridge by movement of the cartridge towards the base of the well and is substantially wholly aspirated.

25

19. Method of performing solid-phase physical, chemical, biological and biochemical techniques comprising the steps of: charging a well having a sump at its base with a sample solution; inserting a hollow plunger into the well, the plunger containing a particulate material and having a nozzle at
30 one end in communication with the interior of the plunger; forming a sliding seal between the plunger and the well; and moving the plunger towards the base of the well, the interior of the sump being conformed to closely fit

about the nozzle whereby small volumes of liquid are drawn from the sump into the plunger by movement of the plunger towards the base of the well and are substantially wholly aspirated.

- 5 20. Method as claimed in claim 19, wherein the steps are repeated with at least one further solution with the same plunger being used in each step.

21. Method as claimed in claim 20, wherein solid-phase extraction is performed.

10

22. Method as claimed in claim 20, wherein the steps are performed to introduce a chemical grouping to the particulate material and the steps are then repeated at least once to force a reaction solution through the particulate material including the chemical grouping.

15

23. Method as claimed in claim 20, wherein a specific affinity species is introduced into the particulate material.

24. Method as claimed in claim 20, wherein at least one enzyme is
20 introduced into the particulate material.

25. Method of performing chromatographic separation comprising the steps of:

- charging a well having a sump at its base with a liquid;
25 introducing a cartridge containing chromatographic material into the well, the cartridge having a nozzle at one end in communication with the interior of the cartridge;
 forming a seal between the sides of the cartridge and the walls of the well;
30 driving the cartridge from a first upper position in the well to a second lower position within the well thereby forcing the liquid to pass through the chromatographic material; and

selectively collecting liquid from above the chromatographic material.

AMENDED SHEET (ARTICLE 19)

1/6

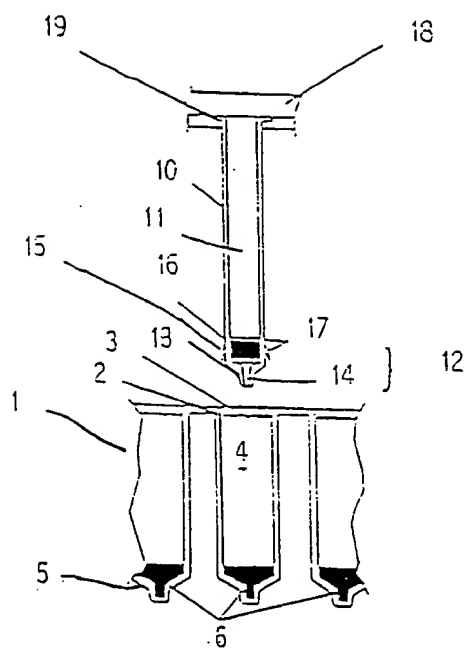
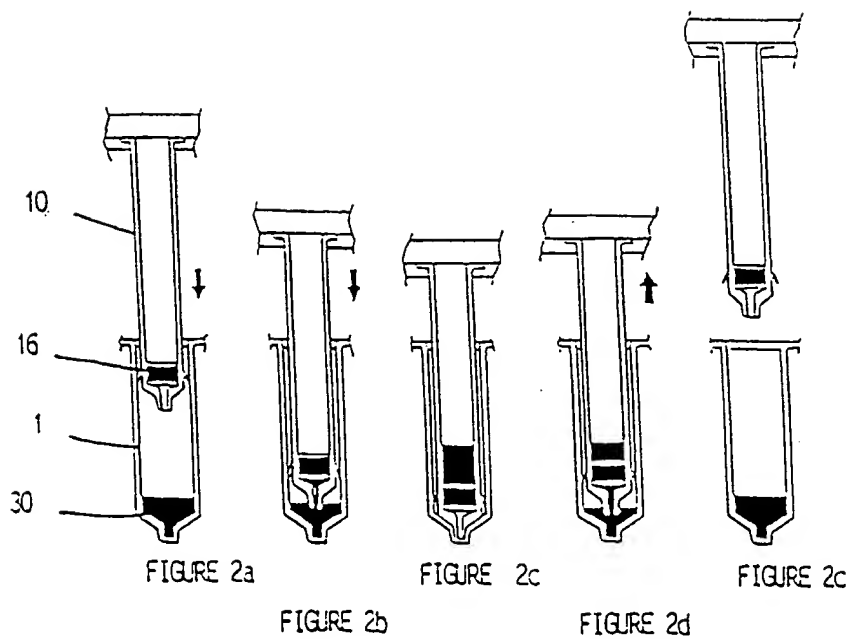


FIGURE 1



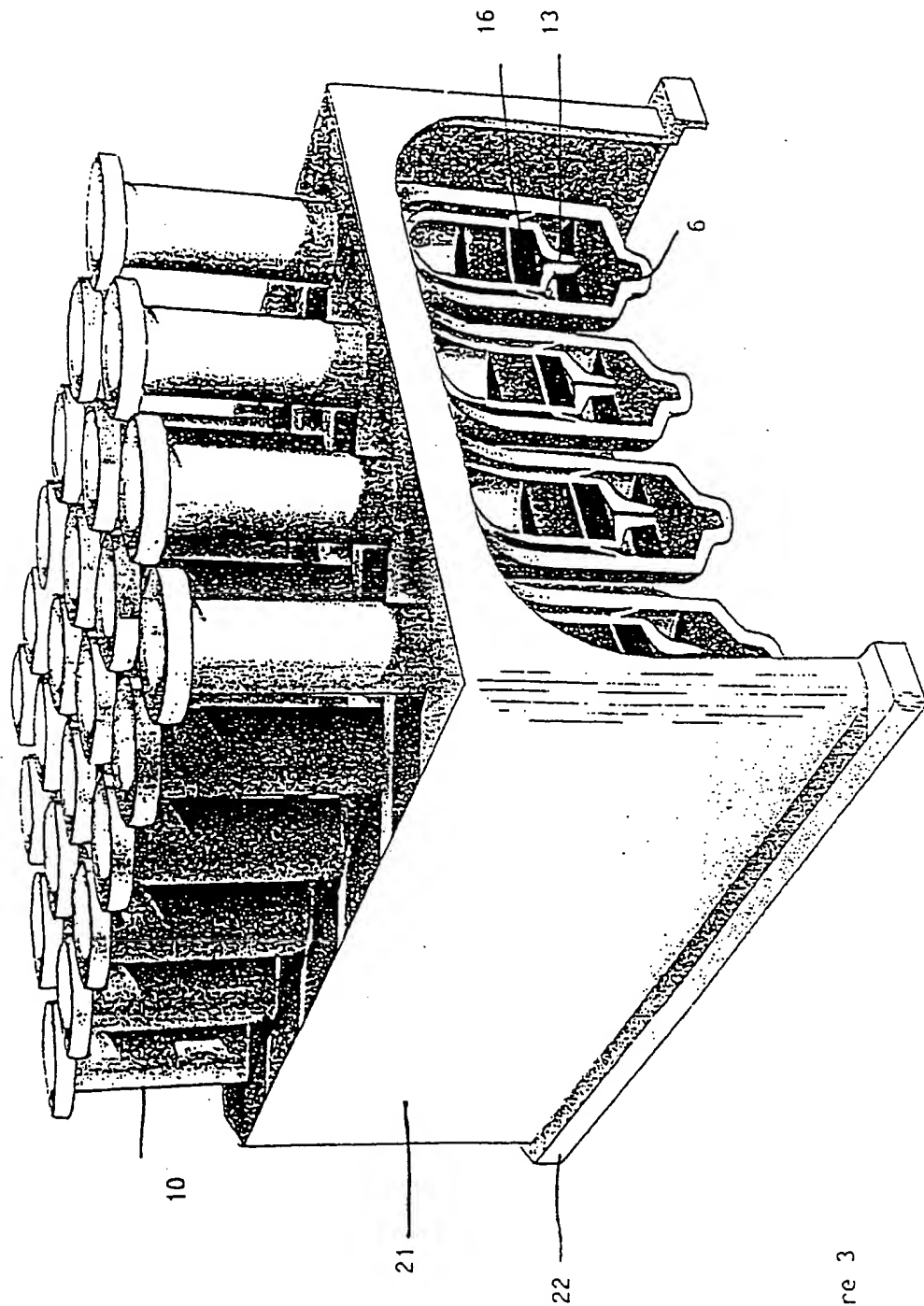


Figure 3

3/6

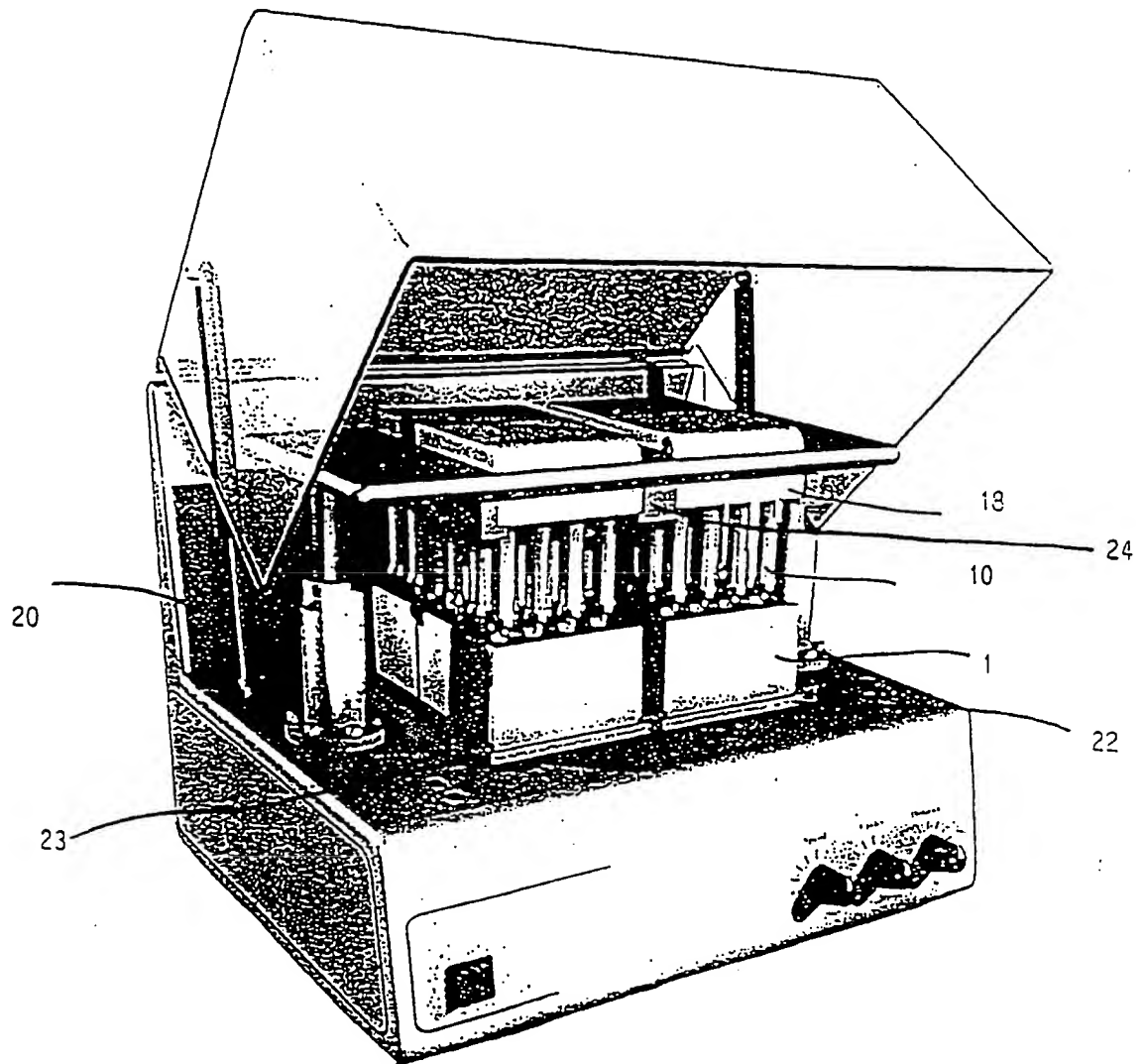


Figure 4

SUBSTITUTE SHEET (RULE 26)

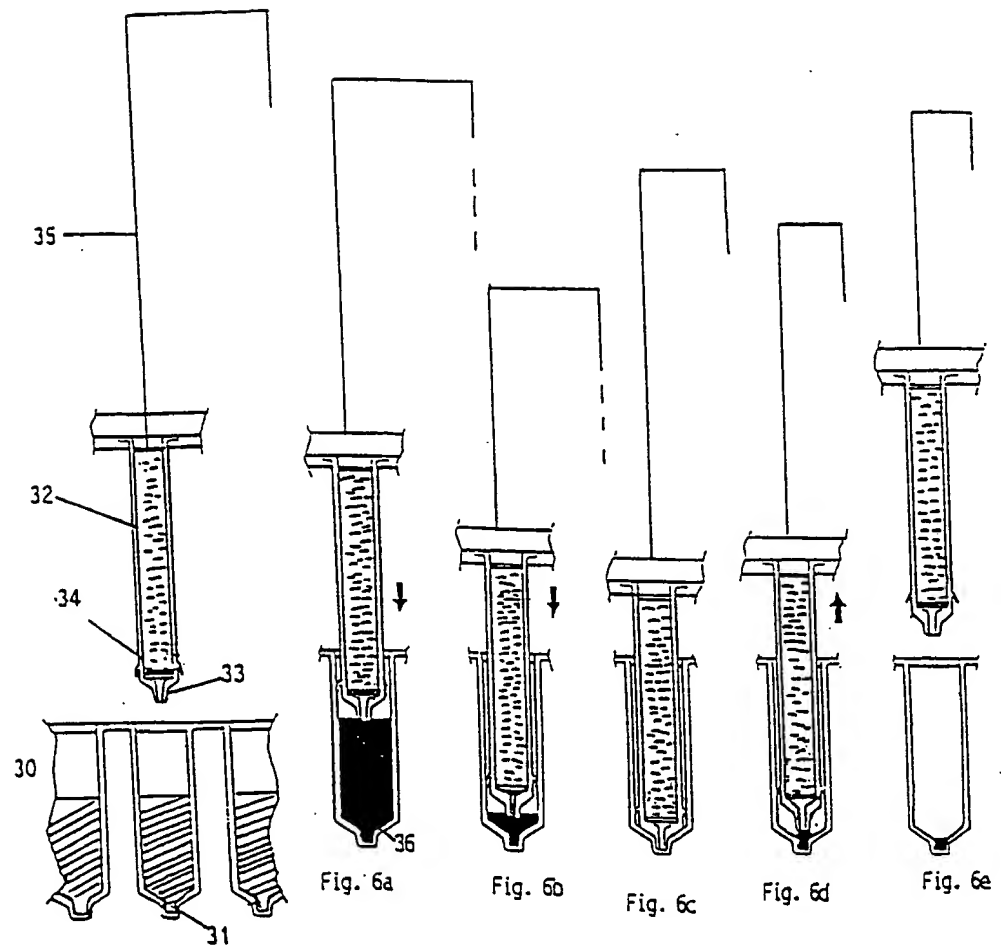
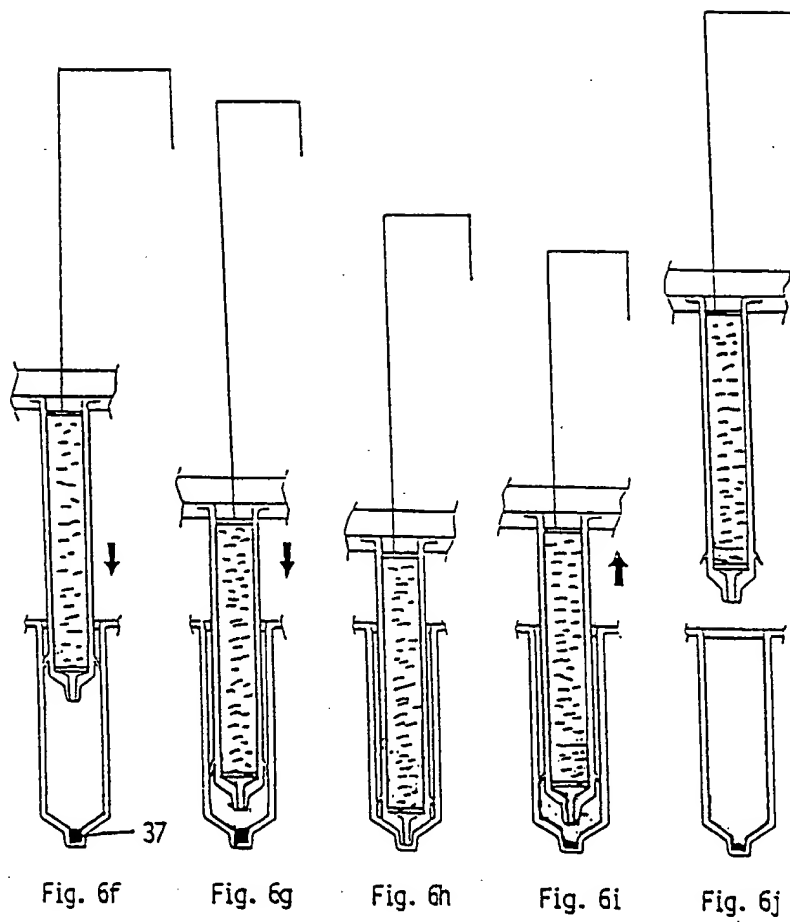
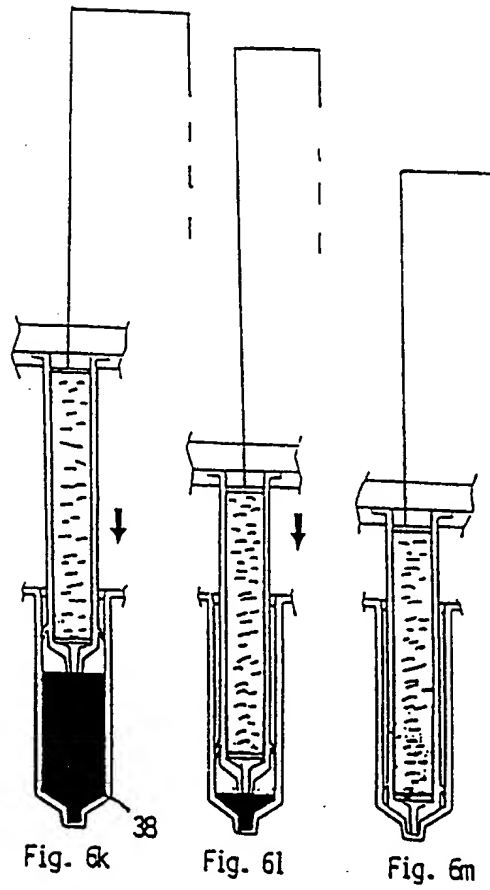


Figure 5





INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 97/03155

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 B01J19/00 B01D15/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 B01J B01D G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 424 279 A (JOSEPH W. BOHN ET AL.) 3 January 1984 see abstract see column 4, line 5 - line 67 see column 10, line 8 - column 12, line 23 see figures	1,8,10, 14-16
A	---	9,13,19, 21,26,28
	-/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

16 February 1998

Date of mailing of the international search report

25/02/1998

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INTERNATIONAL SEARCH REPORT

Int. Patent Application No

PCT/GB 97/03155

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
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Y	---	5-7, 17, 22, 26
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A		4, 9, 21, 26
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